Withholding parenteral nutrition during critical illness increases plasma bilirubin but lowers the incidence of biliary sludge

AUTHORS:

Yoo-Mee Vanwijngaerden[1,*] (yoo-mee.vanwijngaerden@med.kuleuven.be)

Lies Langouche[1,*] (lies.langouche@med.kuleuven.be)

Richard Brunner[1] (richard.brunner@med.kuleuven.be)

Yves Debaveye[1], (yves.debaveye@uzleuven.be)

Marijke Gielen[1] (marijke.gielen@uzleuven.be)

Michael Casaer[1] (michael.casaer@uzleuven.be)

Christopher Liddle[2] (chris.liddle@sydney.edu.au)

Sally Coulter[2] (sally.coulter@sydney.edu.au)

Pieter J. Wouters[1] (pieter.wouters@uzleuven.be)

Alexander Wilmer[3] (alexander.wilmer@uzleuven.be)

Greet Van den Berghe[1] (greet.vandenberghe@med.kuleuven.be)

Dieter Mesotten[1] (dieter.mesotten@med.kuleuven.be)

[1] University Hospitals of the KU Leuven, Intensive Care Medicine and Department of Molecular and Cellular Medicine, Leuven, Belgium

[2] Storr Liver Unit, Westmead Millennium Institute and University of Sydney, Westmead Hospital, Westmead NSW, Australia

[3] University Hospitals of the KU Leuven, Medical Intensive Care Unit, Leuven, Belgium

*YV and LL contributed equally to the manuscript

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CORRESPONDENCE:

Dieter Mesotten, MD, PhD

University Hospitals of the KU Leuven, Intensive Care Medicine

Department of Molecular and Cellular Medicine KULeuven

Herestraat 49, B-3000 Leuven

Belgium

Tel: +32-16 34 40 21, Fax: +32-16 34 40 15, Email: dieter.mesotten@med.kuleuven.be

LIST OF ABBREVIATIONS:

CLD cholestatic liver dysfunction, GBS gallbladder sludge, CI critical illness, PN parenteral nutrition, ICU intensive care unit, ALT alanine aminotransferase, AST aspartate aminotransferase, GGT gamma-glutamyl transpeptidase, ALP alkaline phosphatase, BA bile acid, EPaNIC Impact of Early Parenteral Nutrition Completing Enteral Nutrition in Adult Critically Ill Patients, DPD 2,5-dichlorophenyldiazonium, IFCC International Federation of Clinical Chemistry, AMP adenosine monophosphate, AROC area under the receiver operating characteristic curve, ULN upper limit of normality, SOFA Sequential Organ Failure Assessment, MOD multiple organ dysfunction, SAPS Simplified Acute Physiology Score

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Abstract

Cholestatic liver dysfunction (CLD) and biliary sludge often occur during critical illness and are allegedly aggravated by parenteral nutrition (PN). Delaying initiation of PN beyond day 7 in ICU (late-PN) accelerated recovery as compared with early initiation of PN (early-PN). However, the impact of nutritional strategy on biliary sludge and CLD has not been fully characterized.

This was a preplanned subanalysis of a large RCT of early-PN versus late-PN (n=4640). In all patients plasma bilirubin (daily) and liver enzymes (ALT/AST/GGT/ALP; twice weekly; n=3216) were quantified. In a random predefined subset of patients also plasma bile acids (BAs) were quantified at baseline and on days 3, 5 and last ICU-day (n=280). Biliary sludge was ultrasonographically evaluated on ICU-day 5 (n=776).

From day 1 after randomization until the end of the 7-day intervention window, bilirubin was higher in the late-PN than in the early-PN group ($p<0.001$). In the late-PN group, as soon as PN was started on day 8, bilirubin fell and the two groups became comparable. Maximum levels of GGT, ALP and ALT were lower in the late-PN group ($p<0.01$). Glycine/taurine-conjugated primary BAs increased over time in ICU ($p<0.01$), similarly for the two groups. Fewer patients in the late-PN than in the early-PN group developed biliary sludge on day 5 (37% vs 45%; $p=0.04$).

In conclusion, tolerating substantial caloric deficit by withholding PN until day 8 of critical illness increased plasma bilirubin but reduced the occurrence of biliary sludge and lowered GGT, ALP and ALT. These results suggest that hyperbilirubinemia during critical illness does not necessarily reflect cholestasis and instead may be an adaptive response that is suppressed by early-PN.

Introduction

Cholestatic liver dysfunction (CLD) – most typically defined as hyperbilirubinemia above 3 mg/dL - occurs in almost 20% of critically ill patients and is an independent risk factor for unfavorable outcome (1-3). Hyperbilirubinemia is a common phenotype of numerous diseases and syndromes in critically ill patients. Mechanical obstruction of the extrahepatic bile duct is easily and robustly diagnosed by ultrasonography, but is only a rare cause of CLD in the intensive care unit (ICU). CLD is predominantly due to intrahepatic non-obstructive cholestasis, which is essentially the inability of the hepatocyte to secrete bile into the bile duct, leading to an intracellular accumulation of bilirubin and bile acids (BAs). Although increased concentrations of BAs inside the hepatocytes are the likely cause of cholestatic liver damage, plasma concentrations of bilirubin, and/or alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) are mostly used in clinical practice for CLD diagnosis (4).

Biliary sludge may also be part of the spectrum of CLD during critical illness due to a lack of bile flow. Notably, critically ill patients with absent oral intake and on total parenteral nutrition are susceptible to the development of biliary sludge. This also applies to patients after gastric surgery or transplantation and patients in whom antibiotics such as ceftriaxone are used (5). The clinical relevance of gallbladder sludge is however unclear and therapeutic interventions are reserved for patients who develop acalculous cholecystitis.

Parenteral nutrition (PN) is assumed to aggravate both CLD and biliary sludge formation (6). In a large randomized controlled multicenter trial we have recently assessed the outcome effect of tolerating a nutritional deficit during the first week in ICU (Late PN), compared with early initiation of PN to supplement insufficient enteral feeding (early PN) (7, 8). Late PN enhanced organ function recovery, reduced the incidence of new infections and shortened duration of ICU stay. Strikingly, more patients in the late PN group had hyperbilirubinemia above 3 mg/dL, whereas fewer late PN patients had a clinically relevant increase in levels of GGT or ALP (8). We therefore hypothesized that increased bilirubin levels during critical illness do not necessarily reflect onset of clinically significant CLD. In

contrast, hyperbilirubinemia may represent an adaptive response, as bilirubin can act as an endogenous anti-oxidant and may counteract the pro-oxidative effects of BA (9-11).

Therefore, the aim of the present study was to compare the effect of late versus early PN on circulating bilirubin, BAs and the liver enzymes GGT, ALP, alanine aminotransferase (ALT), aspartate aminotransferase (AST) over time. In addition, we evaluated the impact of late PN versus early PN on biliary sludge by ultrasonography on ICU day 5.

Material and methods

Study design and patient characteristics

This study was a preplanned prospective subanalysis of a large randomized controlled trial on the effect of early versus late initiation of PN on the outcome of critical illness (EPaNIC trial) (12). The study protocol was previously described in detail (8). Patients were allocated either to the early PN group, where PN was initiated within 48 hours after ICU admission to supplement insufficient enteral nutrition (early PN n=2312) or to the late PN group, where PN was not initiated before day 8 (late PN n=2328) (Figure 1). Baseline characteristics for both allocation groups were comparable in the total study population (Supplemental table 1).

Time course of bilirubin was assessed in all patients while in ICU (early PN n=2312; late PN n=2328). Liver enzymes ALT, AST, GGT and ALP were quantified twice weekly in all patients. Additionally, in 3216 EPaNIC patients (early PN n=1760; late PN n=1794) also plasma conjugated bilirubin was quantified during ICU stay as part of the daily routine clinical practice.

For determination of the plasma levels of conjugated and unconjugated bile acids and conjugated bilirubin, a subgroup of patients, identifiable upon ICU admission, was chosen. Only patients for whom early enteral nutrition was surgically contra-indicated were selected (8). This selection was chosen to prevent possible bias by enterohepatic recycling of BA and bilirubin by enteral feeding. Of the 517 patients in this subgroup (256 patients in the early PN group and 261 patients in the late PN

group) a random selection of 280 patients (140 out of each allocation group) was made to reduce the number of samples for analysis, while testing the hypothesis with enough statistical power. Random selection was performed by a computer algorithm (StatView software, SAS Institute Inc., Cary, North Carolina). Patients in the early and late PN group were comparable for baseline characteristics (Supplemental table 1). In this subgroup, nutritional intake was according to the study protocol (Supplemental figure 1).

For analysis of the impact of early versus late PN on gallbladder sludge, all patients who were still in ICU on the morning of day 5 (early PN n=975, late PN n=913) were eligible for ultrasonography of the gallbladder (Figure 2). Patients with a history of cholecystectomy (n=90), patients who were moribund (n=2), or patients who were discharged from ICU on day 5 before 12 am (n=147) were excluded from the ultrasonography study. Additionally, 783 patients were excluded due to logistical reasons (such as unavailability of ultrasonography device or ultrasound assessor). In total 776 patients (early PN n=420; late PN n=356) underwent ultrasonographical investigation of the gallbladder on day 5 of their ICU stay. Baseline characteristics in both treatment groups were comparable (Supplemental table 1).

Plasma concentrations of bilirubin, liver enzymes and bile acids

Plasma total and conjugated bilirubin were quantified by a standard routine automated laboratory assays (colorimetric DPD-method, HITACHI/Roche for Cobas c702). Liver enzymes ALT, AST, GGT and ALP were quantified by standard routine automated laboratory assays (ALT and AST with the UV kinetic method according to the International Federation of Clinical Chemistry (IFCC), GGT with the Szasz kinetic colorimetric assay and ALP with an AMP kinetic colorimetric assay according to the IFCC, all Hitachi/Roche for Cobas c702).

Plasma concentrations of unconjugated and conjugated primary bile acids cholic acid and chenodeoxycholic acid were measured, as well as the conjugated and the unconjugated secondary

bile acid deoxycholic acid by high performance liquid chromatography-mass spectrometry by using authentic bile acid standards and deuterated internal standards, as previously described (13).

Ultrasonography of the gallbladder

Gallbladder sludge, wall thickness and wall doubling were evaluated by blinded assessors (YV, YD, MG) using ultrasonography. The diagnosis of gallbladder sludge was based on the presence of a low-amplitude echogenic collection layering in the most dependent portion of the gallbladder. A thickened gallbladder wall (> 5mm) and wall doubling (presence of pericholecystic fluid) were assessed at the anterior part of the gallbladder.

Statistical analysis

Statistical analysis was performed with Statview 5.0.1 (SAS Institute Inc., Cary, North Carolina SAS). All quantitative values were assessed for normality. Values with normal distribution, and those that were normalized after logarithmic transformation, were compared with the unpaired and paired Student's t-test. The non-normally distributed data were compared by the non-parametric Mann-Whitney U-test and Wilcoxon signed rank test. Nominal and ordinal variables (expressed as numbers and percentages) were compared with Fisher's exact test. Correlations between variables were calculated using the Pearson's rank correlation test. For all tests a p-value less than 0.05 was deemed significant. To assess the potential predictive power of circulating bilirubin and/or bile acids, area under the receiver operator characteristics curve (AROC) values were calculated with SPSS software (SPSS version 2.0, IBM Corp, New York, USA). Additionally, the relationship between categorized day 1 bilirubin levels and mortality of all EPaNIC patients was plotted.

Results

Effect of late initiation of parenteral nutrition on bilirubin and the other liver enzymes tests

Peak plasma levels of total bilirubin determined during the whole ICU stay and determined during the study intervention window (first 7 days in ICU) were higher in late PN patients in comparison with early PN patients (Table 1). From day 1 after randomization until the end of the 7-days intervention window, also daily plasma total bilirubin was higher in the late PN than in the early PN group (all $p<0.001$) (Figure 3). From day 8 onwards, when PN was commenced in late PN patients and total caloric intake levels became comparable in both groups, plasma total bilirubin was no longer different between late and early PN patients. In the subgroup of patients admitted with sepsis (early PN n=510, late PN n=505) changes were comparable to those in the total population: bilirubin levels were higher in late PN patients from day 2 until day 8 ($p<0.05$) and became similar in the two groups from day 8 onwards. Conjugated bilirubin levels quantified during clinical routine in 3216 EPaNIC patients correlated with total bilirubin ($r>0.900$ and $p<0.01$ for all) and were higher in late PN compared to early PN patients ($p<0.05$ until day 7). Also, peak levels of conjugated bilirubin were higher in late versus early PN patients (Table 1). In the total patient population, conjugated levels represented 80% of total bilirubin.

In contrast with bilirubin, peak levels of GGT were lower in the late PN group compared with the early PN group determined during the total ICU stay as well as during the study intervention window (Table 1). Peak levels of ALP were also lower in the late PN group in comparison with the early PN group, but only when determined over the total ICU stay. Peak levels of the hepatocyte lysis enzyme ALT were lower in the late PN group compared to the early PN group during the total ICU stay as well as during the study intervention window. Peak levels of AST did not differ between the 2 treatment groups (Table 1).

Effect of late initiation of parenteral nutrition on bile acids and conjugated bilirubin

In the subset of patients for whom early enteral nutrition was surgically contra-indicated, circulating BAs were determined by mass-spectrometry. In these patients, compared with the admission values, the circulating glycine- and taurine-conjugated primary BAs, cholic acid and chenodeoxycholic acid,

were elevated on day 3 and day 5 of ICU stay (Figure 4). Also, the unconjugated primary BA cholic acid was mildly increased on day 3 and day 5. Remarkably, the concentration of conjugated cholic acid was 5- to 50-fold higher than the levels of unconjugated cholic acid ($p<0.01$ for all – Figure 4). The secondary BA, deoxycholic acid, either unconjugated or conjugated, did not change over time in these critically ill patients. The levels of circulating bile acids were comparable in early and late PN patients. In this subgroup of patients, conjugated bilirubin levels correlated well with the total bilirubin levels on admission, on day 3 and day 5. They were higher in the late PN patients compared to the early PN patients (Figure 5). Of the total bilirubin levels, 35% was conjugated.

Effect of late initiation of parenteral nutrition on gallbladder sludge

Fewer patients in the late PN group developed gallbladder sludge than in the early PN group (37% versus 45%; $p=0.04$)(Table 2). Also the incidence of double gallbladder wall tended to be lower in the late PN group in comparison with the early PN group (3.3% versus 6.1%, $p=0.08$). The incidence of wall thickening was comparable for both groups (5.7% versus. 6.2%, $p=0.8$).

Predictive value of bilirubin and bile acids for ICU mortality

The relationship between day 1 bilirubin levels and ICU mortality displays a “hockey stick” shape (Figure 6): very low levels of bilirubin (<0.36 mg/dL) were associated with a mild increase in mortality risk while patients with normal to mildly elevated bilirubin levels (0.36 – 2.39 mg/dL) displayed the lowest mortality rates. Only in patients with day 1 bilirubin exceeding 2.40 mg/dL was the ICU mortality risk sharply increased. The AROC curve value for day 1 bilirubin to predict ICU mortality was 0.597 (calculated in the total study population of 4640 patients). The AROC values of admission BAs to predict ICU mortality ranged from 0.455 (tauro-deoxycholic acid) to 0.631 (tauro-chenodeoxycholic acid), calculated in the bile acid study population (n=280).

Discussion

This study demonstrated that early initiation of parenteral nutrition in critically ill patients immediately suppressed plasma bilirubin concentrations. This effect of early PN occurred without affecting the plasma concentrations of circulating bile acids. Early PN during critical illness increased the levels of cholestatic liver enzymes GGT and ALP and increased the incidence of biliary sludge. The latter is in line with the previously observed association between long-term administration of PN and the development of biliary sludge in critically ill patients (14) and with the results from a rabbit experiment documenting gallbladder distension within 1 week of PN (15). However, the association between PN and biliary sludge in the critical care setting has always been biased by the fact that more severely ill patients do not tolerate enteral feeding and rely on PN to maintain caloric intake. With the current study, a causal relationship was established. We demonstrated that postponing the administration of PN reduced the incidence of gallbladder sludge during critical illness. Biliary sludge thus appears to be in part a preventable complication of critical illness as metabolic interventions such as tolerating caloric deficits by late PN but also tight blood glucose control (2) synergistically lowered the occurrence of gallbladder sludge. Whether the caloric restriction is bringing about this effect directly or indirectly through the decreased incidence of ICU acquired infections (8) and lowered antibiotic use (16) cannot be delineated from this study.

We previously reported that avoiding early administration of PN during the first week lowered the proportion of patients with GGT and ALP levels above 1.5 x upper limit of normality (ULN), our a priori definition of cholestasis during ICU stay (2, 8). While average peak levels of GGT and ALP were higher in the early versus late PN group, peak levels still remained below this 1.5 x ULN cut-off. Similar subtle differences were seen in plasma ALT levels. While mean levels were higher in the early PN group, the proportion of patients with ALT > 3 x ULN did not differ between the treatment groups (8). Taken together, this indicates that overt cholestasis, as seen in chronic TPN administration and hyperalimentation, is not frequent in critically ill patients. However, early administration of PN may

not be well tolerated by the liver during critical illness, causing a relatively mild elevation of liver enzymes (6).

In contrast with the liver enzymes, peak total bilirubin levels, the archetypical biochemical marker of cholestasis during critical illness, remained lower in the patients exposed to the early administration of PN. Conversely, patients in the late PN group consistently revealed higher bilirubin levels coinciding with a better outcome (shorter ICU stay and less ICU-acquired infections). As soon as PN was started after 1 week in the late PN group, this difference was mitigated. It confirms our previous finding that “cholestasis” defined by bilirubin levels > 3 x ULN is more frequent in the late PN group during the intervention window (8). Hence, a rise in plasma blilirubin may be an adaptive response in the context of caloric restriction during critical illness. Also, in other population studies elevated bilirubin levels had a protective effect against coronary artery disease (17) and stroke (18). Hyperbilirubinemia may exert its beneficial effect by improving endothelial function and decreasing oxidative stress (19). Heme oxygenase-1, which is the rate-limiting enzyme in the bilirubin production, is protective in endothelial cells against toxicity associated with high glucose and oxidative stress (20). Heme oxygenase knock-out mice have been demonstrated to have a higher mortality and more liver and kidney injury during endotoxic shock (21). Additionally, bilirubin administration was shown to attenuate liver and kidney damage in animal models (22, 23).

However, in this study peak bilirubin levels were associated with ICU mortality risk in a “hockey stick” relationship. Very low levels of bilirubin are associated with a mild increase in mortality risk. Hyperbilirubinemia on admission only correlated with increased mortality risk when levels exceed 2.5 -3.0 x ULN. This may explain why bilirubin, analyzed as a continuous variable, has a poor predictive power for mortality in non-cirrhotic critically patients (24). Nevertheless, peak bilirubin levels are still used in the most prevalent scoring systems for organ failure such as the SOFA (25), MOD (26) and SAPS score (27). Importantly, peak plasma concentration of bilirubin or GGT/ALP do not seem interchangeable in the definition of overt cholestasis (2, 6, 28). GGT/ALP may be the better indicators

for cholestasis during critical illness, as these enzymes are believed to reflect “cholate stasis” in the bile canaliculi (29). Alternatively, a higher cut-off for bilirubin as a marker of CLD should be clinically validated.

We also observed that the bile acid profile was affected by critical illness, but not by the timing of PN initiation. In contrast to Recknagel et al. (24), in our study population, plasma concentration of BAs did not predict mortality of critically ill patients better than did plasma bilirubin. The conjugated primary bile acids, cholic acid and chenodeoxycholic acid, increased over time while the concentrations of unconjugated bile acids were not significantly altered during the course of critical illness. Also the conjugated secondary bile acid, deoxycholic acid, was unchanged. This lack of impact on circulating BAs may partially be explained by the selection of patients with a surgical contraindication to enteral feeding. Consequently, the enterohepatic BA cycle may have been impaired. Whether enteral nutrition would exert an additional effect on circulating BAs, cannot be addressed as the study was not randomized for enteral nutrition and the specific subset of patients for whom BAs were quantified, did not receive any enteral nutrition. Similarly, in the subset of patients with a contraindication to enteral feeding, the proportion of conjugated bilirubin was much lower than in the overall patient population. The mechanism of this conjugation deficit is not clear and may be related to the higher severity of illness observed in the patient population with a contra-indication to enteral nutrition. However, unconjugated bilirubin may have more potent anti-oxidative properties (30). Therefore, the mild unconjugated hyperbilirubinemia early in the course of critical illness may be a different entity to the pronounced conjugated hyperbilirubinemia typical of prolonged critically ill patients, the so called “ICU jaundice” (31). Nevertheless, the dual role of bilirubin during critical illness remains associative from our study data. Only an interventional study, in which circulating levels of bilirubin are actively manipulated, can answer whether mild hyperbilirubinemia truly protects the patient during critical illness.

In conclusion, withholding PN and accepting a large caloric deficit during the first week of critical illness increased plasma concentrations of bilirubin, lowered plasma levels of GGT and ALP but not of BAs, and reduced the incidence of gallbladder sludge. These results suggest that hyperbilirubinemia during critical illness does not necessarily reflect cholestasis. Instead hyperbilirubinemia may be an adaptive response, which is suppressed by early PN.

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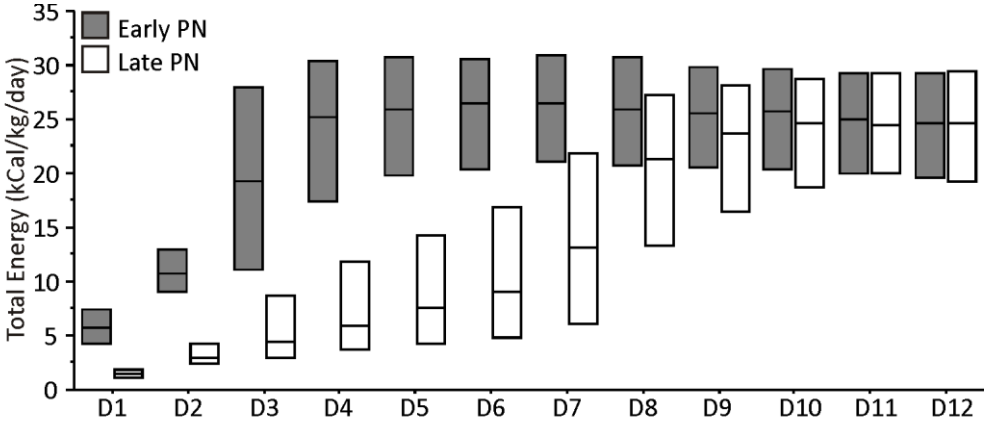
Figure legends



Early PN	2312	1833	1438	1185	975	848	736	646	601	562	517	479
Late PN	2328	1885	1399	1117	913	760	655	574	524	481	436	397

Figure 1: Total energy intake levels during the EPaNIC study. Boxes represent daily total caloric intake (glucose, lipids and proteins) expressed as medians with IQR (25^{th}-75^{th} percentiles). The grey boxes represent daily caloric intake of patients randomized to receiving early parenteral nutrition (early PN), whereas open boxes are presenting values from patients randomized to nutrient restriction (late PN). The number of patients still in ICU is plotted for each day. Abbreviations: IQR interquartile range, PN parenteral nutrition, ICU intensive care unit.

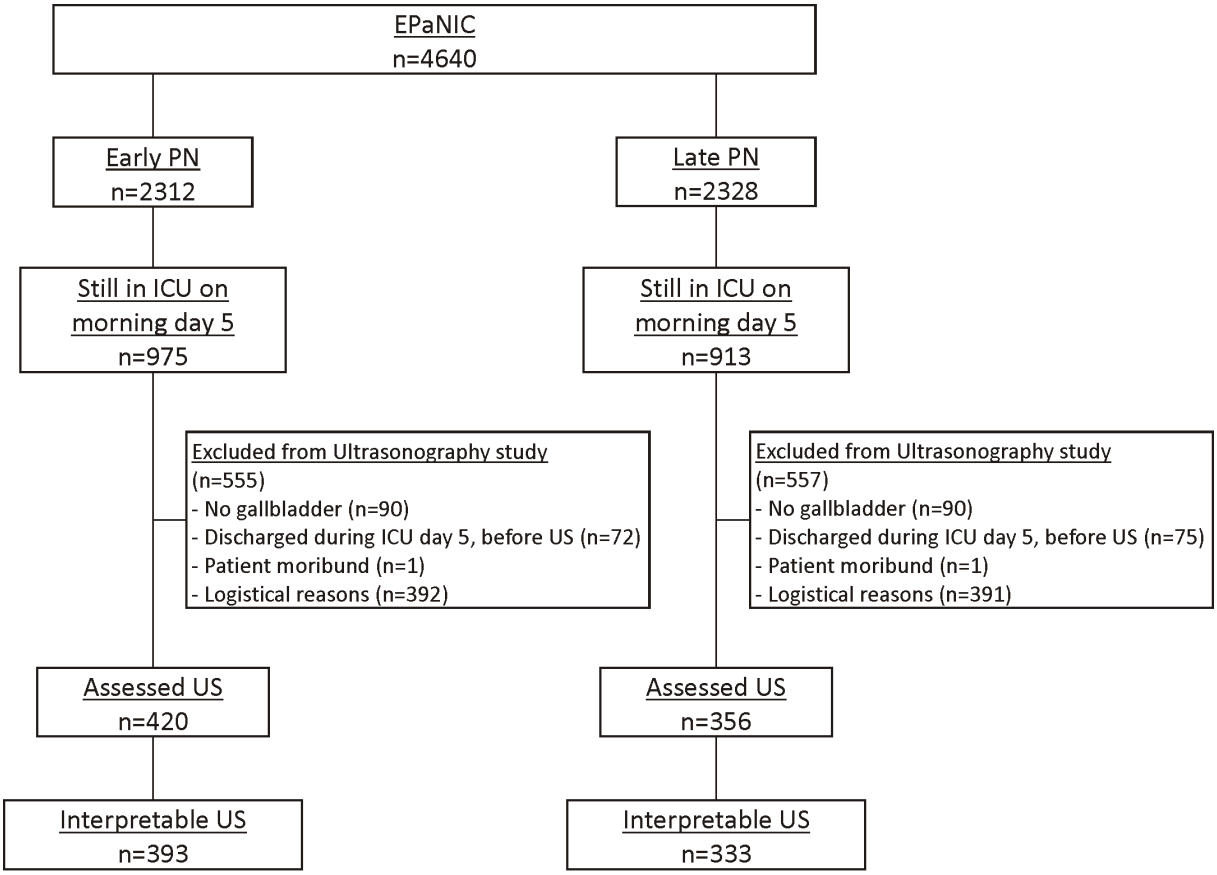


Figure 2: Consortdiagram of enrollment for the ultrasonography study. Only patients with an ICU stay of at least 5 days were selected for evaluation. Abbreviations: PN parenteral nutrition, ICU intensive care unit, US ultrasonography.

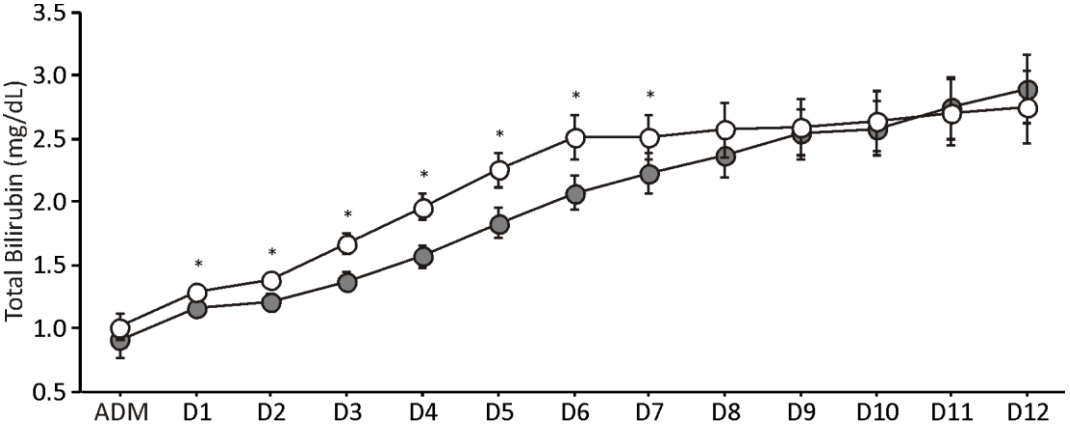


Figure 3: Daily plasma total bilirubin levels. Daily plasma total bilirubin levels of all patients still in ICU are presented as mean ± standard error of the mean. The grey dots present values of early PN patients, whereas open dots are presenting values from late PN patients. * p≤0.05 with the unpaired Student's t-test after logarithmic transformation. Abbreviations: PN parenteral nutrition, ADM admission.

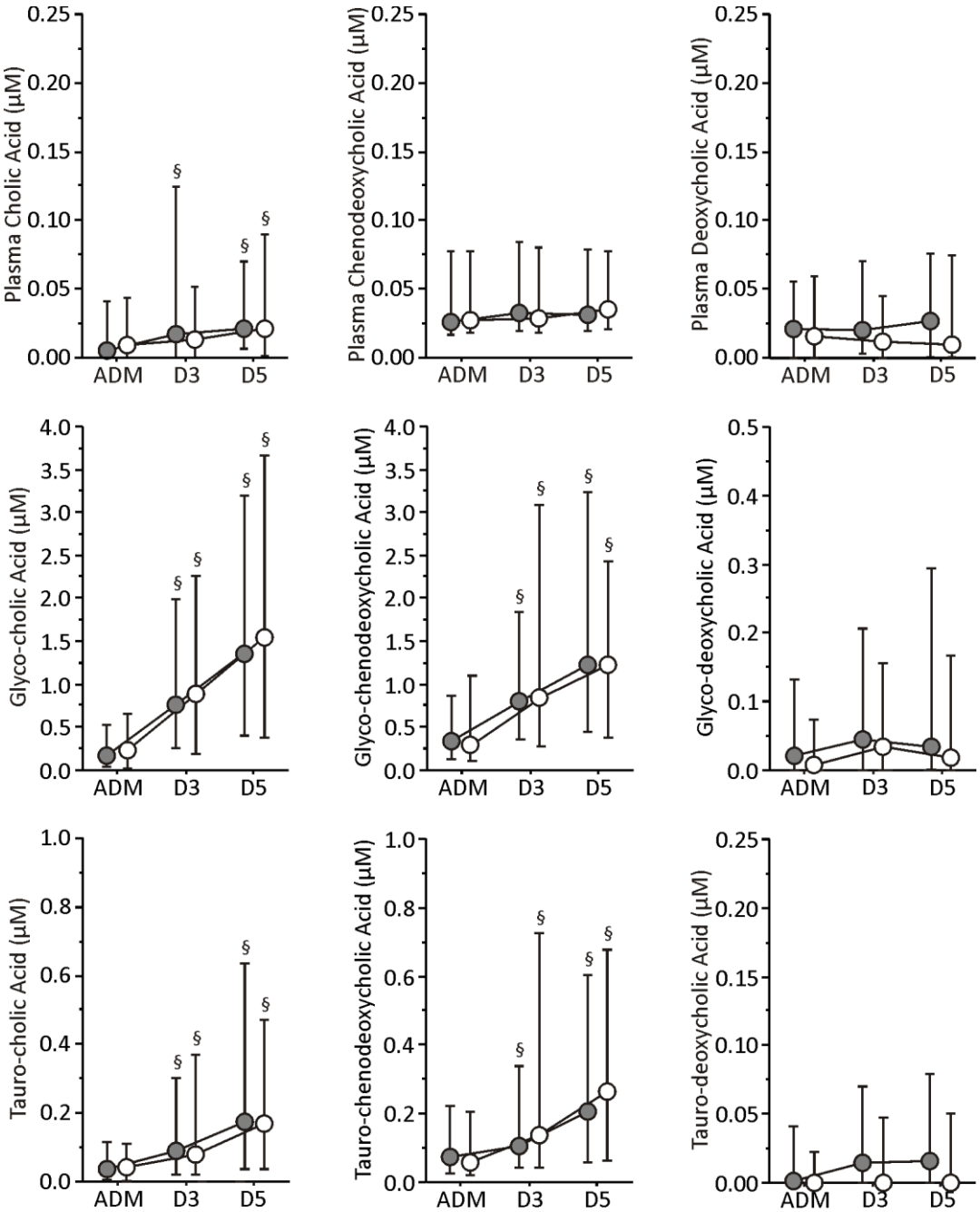


Figure 4: Circulating plasma levels of bile acids. Plasma levels of bile acids (expressed in µM) on admission, on day 3 and day 5 of ICU stay are represented as median with IQR (25^{th}-75^{th} percentiles). The grey dots present values of early PN patients (n=140), whereas open dots are presenting values from late PN patients (n=140). § $p \leq 0.05$ using Wilcoxon rank test for comparison with admission values. Abbreviations: ADM admission, IQR interquartile range, PN parenteral nutrition

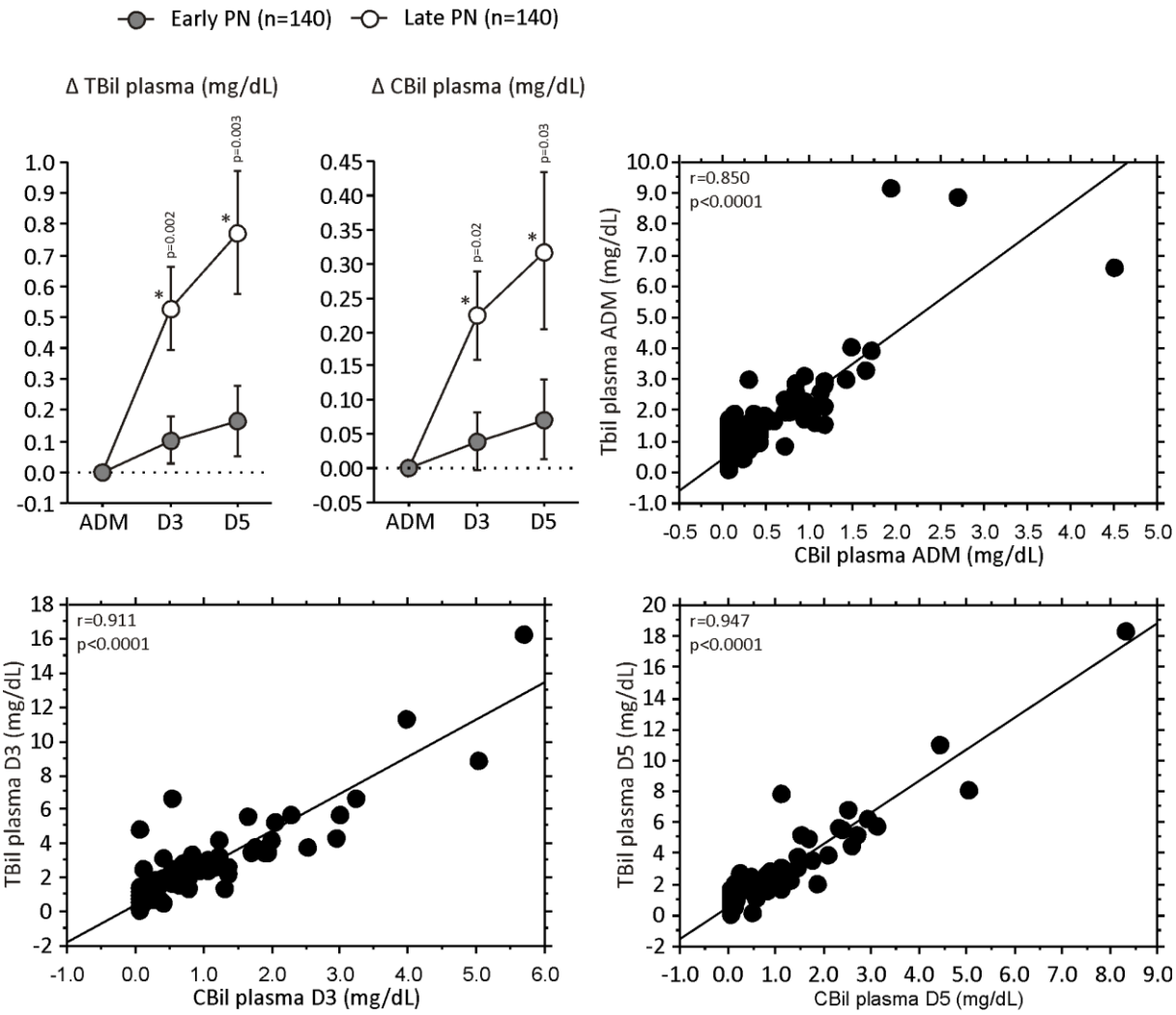


Figure 5: Effect of early versus late initiation of parenteral nutrition on total and conjugated bilirubin levels. The line charts (mean ± SEM) represent the changes from the admission values (referred to as Δ) to day 3 in ICU and to day 5 in ICU in plasma total bilirubin and conjugated bilirubin. The grey dots represent the values of early PN patients, whereas the open dots are presenting values from late PN patients. * p≤0.05 using the Wilcoxon signed rank test for calculations of changes over time; p-values for calculations of differences between early and late PN values obtained using Mann-Whitney U test. Correlations between plasma total bilirubin levels and conjugated bilirubin levels on admission, on day 3 and day 5 of ICU stay are calculated using Pearson's correlation test. Abbreviations: SEM standard error of the mean, ICU intensive care unit, TBil total bilirubin, CBil conjugated bilirubin, ADM admission

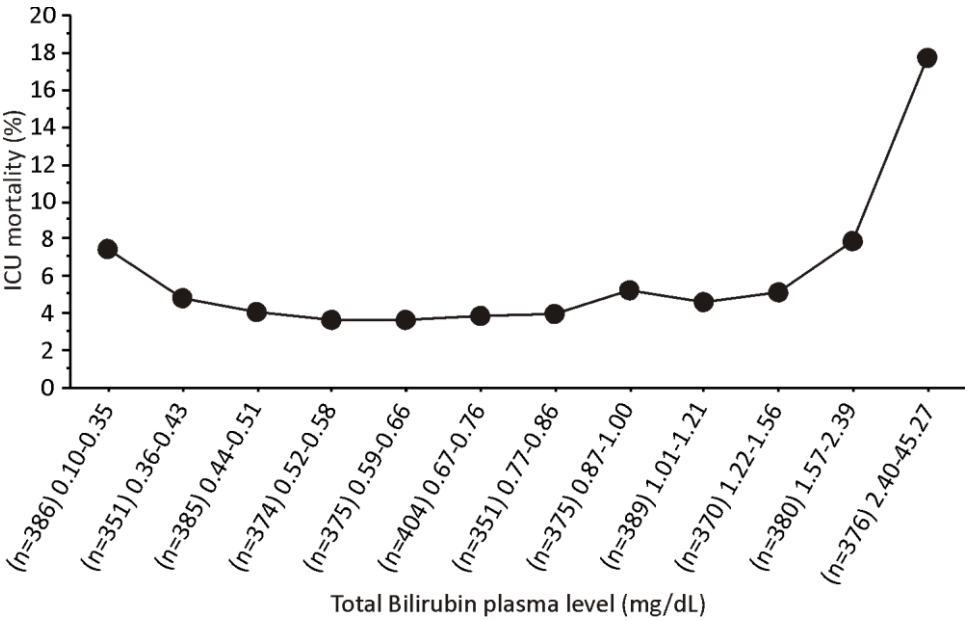


Figure 6: *Relationship between plasma total bilirubin levels on day 1 and ICU mortality.*

Abbreviations: ICU intensive care unit

Table legends

Table 1: Effect of early versus late parenteral nutrition on peak plasma concentrations of total bilirubin and liver enzymes

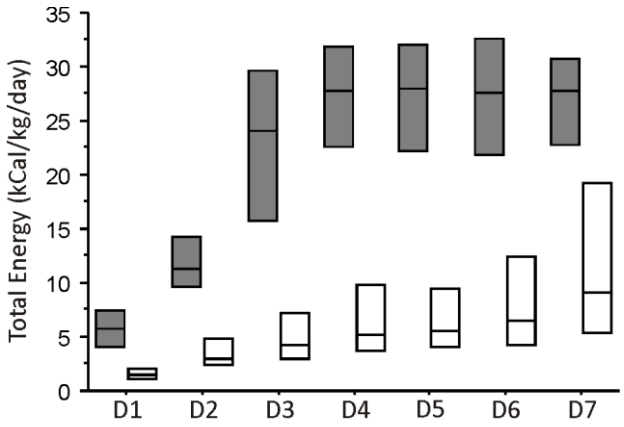
	ICU D1-LD			ICU D1-D8		
	Early PN	Late PN	p-value	Early PN	Late PN	p-value
T Bilirubin (mg/dL)	0.91 [0.60-1.64]	1.01 [0.67-1.78]	< 0.0001	0.87 [0.59-1.48]	0.97 [0.65-1.66]	< 0.0001
C Bilirubin (mg/dL)	0.39 [0.21-1.01]	0.45 [0.24-1.16]	0.0003	0.37 [0.20-0.85]	0.43 [0.24-1.02]	< 0.0001
GGT (IU/L)	50 [19-140]	38 [18-115]	0.0007	40 [18-99]	35 [17-84]	0.002
ALP (IU/L)	178 [102-373]	159 [99-332]	0.02	155 [100-275]	149 [97-257]	0.1
ALT (IU/L)	28 [16-79]	24 [14-65]	0.0003	23 [14-51]	21 [14-45]	0.005
AST (IU/L)	55 [34-106]	55 [33-104]	0.3	50 [32-87]	50 [32- 92]	0.9

Peak plasma concentrations of total bilirubin and liver enzymes for the total EPaNIC study ICU stay and for the EPaNIC study intervention time window (first 8 days of ICU stay) are presented as median [IQR]. P-values are calculated with Mann-Whitney U test. Abbreviations: ICU intensive care unit, C conjugated, T total, GGT gamma-glutamyl transferase, ALP alkaline phosphatase, ALT alanine aminotransferase, AST aspartate aminotransferase

Table 2: Ultrasonography study of the gallbladder

	Early PN (n=420)	Late PN (n=356)	p-value
Sludge – n(%)	175 (44.8)	124 (37.3)	0.04
Wall thickening - n(%)	24 (6.2)	19 (5.7)	0.8
Double wall - n(%)	24 (6.1)	11 (3.3)	0.08

Supplemental Figure 1: Total energy intake levels during the EPaNIC study for the subgroup of patients selected for analysis of bile acids



Early PN	140	121	109	100	92	86	77
Late PN	140	121	96	88	80	70	68

Boxes represents daily total caloric intake (glucose, lipids and proteins) expressed as medians with IQR (25^{th}-75^{th} percentiles). The grey bars represent daily caloric intake of patients randomized to receiving early parenteral nutrition (early PN), whereas open bars are presenting values from patients randomized to nutrient restriction (late PN). The number of patients still in ICU is plotted for each day. Abbreviations: IQR interquartile range, PN parenteral nutrition, ICU intensive care unit.

Suplemental table 1: Baseline characteristics

Patient characteristic	**Total population**			**Subgroup bile acids**			**Subgroup ultrasonography**		
	Early PN n=2312	Late PN n=2328	p-value	Early P**N** n=140	Late PN n=140	p-value	Early PN n=420	Late PN n=356	p-value
Male sex — n (%)	1486 (64)	1486 (64)	0.8	87 (62)	87 (62)	>0.9	280 (67)	230 (65)	0.5
Age - yr	64 ± 14	64 ± 15	0.5	63 ± 14	62 ± 15	0.7	63 ± 15	63 ± 16	0.8
Weight - kg	76 ±16	75 ± 15	0.05	74 ±17	73 ± 17	0.8	75 ±15	75 ± 16	0.6
BMI – kg/m^{2-} n(%)			0.3			0.5			0.6
<20	134 (6)	141 (6)		12 (9)	20 (14)		28 (7)	27 (8)	
20 to <25	854 (37)	890 (38)		65 (46)	55 (39)		172 (4)	139 (39)	
25 to <30	852 (37)	864 (38)		41 (29)	42 (30)		136 (32)	129 (36)	
30 to <40	430 (19)	405 (17)		19 (14)	21 (15)		75 (18)	57 (16)	
≥40	42 (2)	28 (1)		3 (2)	2 (1)		9 (2)	4 (1)	
Diabetes mellitus – n (%)	391 (17)	417 (18)	0.4	17 (12)	19 (14)	0.7	65 (15)	58 (16)	0.8
Malignancy - n (%)	437 (19)	457 (20)	0.9	86 (61)	83 (59)	0.7	97 (23)	84 (24)	0.9
NRS score	4 [3-4]	4 [3-4]	0.3	4 [3-5]	4 [3-5]	0.4	4 [3-5]	4 [3-5]	0.9
Sepsis - n (%)	510 (22)	505 (22)	0.8	80 (57)	68 (49)	0.2	189 (45)	171 (48)	0.4
Emergency admission - n (%)	956 (41)	970 (42)	0.8	89 (64)	92 (66)	0.7	306 (73)	266 (75)	0.6
APACHE II score	23 ± 11	23 ± 10	0.8	27 ± 11	27 ± 11	0.7	31 ± 10	31 ± 9	0.9

Baseline characteristics of the patients of the total study population, the subgroup of patients selected for analysis of bile acids and the subgroup of patients who underwent ultrasonographical evaluation of the gallbladder. Scores from the Acute Physiology and Chronic Health Evaluation II (APACHE II) reflects severity of illness and can range from 0 to 71, with higher scores indicating a greater severity of illness (32). Scores from Nutritional Risk Screening (NRS) range from 0 to 7, with higher scores indicating a higher risk of malnutrition (33). NRS scores are presented as medians with IQR between square brackets. Plus-minus values are means ± SD. Percentages may not total 100 because of rounding. Abbreviations: BMI body mass index, SD standard deviation, IQR interquartile range.

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